Section II (Remarks)

A. Summary of Amendment to the Claims

Claims 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, and 46-48 have been amended as set forth in the above Complete Listing of the Claims. As amended, the claims are supported by the specification and the original claims. No new matter has been added, as defined by 35 U.S.C. § 132.

The amendments made herein are fully consistent with and supported by the originally-filed disclosure of this application.

By the present amendment, cancellation of claims 2, 5, 8, 14, and 62 is requested, without prejudice.

Thus, upon entry of the amendments, claims 1, 3, 4, 6, 7, 9-13, and 15-61 will be pending, of which claims 18-45, 50 and 52-61 are withdrawn. Accordingly, claims 1, 3, 4, 6, 7, 9-13, 15-17, and 46-49 remain pending and under examination in the present application.

B. Rejection Under 35 U.S.C. §112 – Indefiniteness

In the Office Action mailed August 17, 2009 the examiner has maintained the rejection of claims 3, 7 and 13 under 35 U.S.C. §112, second paragraph as indefinite. Applicant respectfully disagrees.

In the Response submitted February 27, 2009, Applicant provided support for the position that the claims were definite, based on the fact that the claimed protease prodomain proteins are described in the specification by both structural and functional characteristics and that, by such description, one of skill in the art would be able to generate a modified protease prodomain protein with increased binding affinity for subtilisin or a variant thereof. Applicant's position was further supported by the fact that the specification clearly describes subtilisin and its variants, including subtilisin-like proteases, and Applicant demonstrated that such a class of proteases has a widely recognized and consistent mechanistic and structural definition that is known and recognized by those of skill in the art. These positions are presently maintained by Applicant.

In response, in the Office Action mailed August 17, 2009, the examiner alleged that "the claim amendments fall short of indicating any structural class of protease prodomains…" and that "…a further amendment to introduce the terms 'a subtilisin protease prodomain'…would not permit the artisan and the public, seeking to determine the scope of the intended subject matter to distinguish between a subtilisin protease prodomain that is not a variant from a subtilisin prodomain that is a variant having…'increased affinity.'"

In order to advance prosecution of the subject application, Applicant has amended claim 1, from which claim 3 depends, claim 3, claim 7 and claim 13. By the amendments to the claims, the prodomain protein has been amended to specify a subtilisin prodomain protein and the specific protease to which it binds is recited as a subtilisin or variant thereof.

As previously demonstrated to the examiner, protease prodomain proteins, in particular subtilisin prodomain proteins, are described in detail throughout the specification and are particularly defined at page 14, line 31 to page 15, line 6:

> The term "protease prodomain protein" refers to prodomain amino acid sequence or functional equivalent thereof wherein the protease prodomain protein possesses the capability of binding to a corresponding protease with high affinity. Preferably, the prodomain is substantially free of other proteins with which it is naturally associated, for instance, the balance of the protease protein. In addition, one or more predetermined amino acid residues in the prodomain may be substituted, inserted, or deleted, for example, to produce a prodomain protein having improved biological properties, or to vary binding and expression levels. Through the use of recombinant DNA technology, the prodomain proteins of the present invention having residue deletions, substitutions and/or insertions may be prepared by altering the underlying nucleic acid.

Furthermore, the subtilisin prodomain proteins are described at page 13, lines 1-14:

> The isolated subtilisin prodomain is unfolded but assumes a compact structure with a four-stranded anti parallel β-sheet and two three-turn α-helices in complex with subtilisin… The C-terminal residues extend out from the central part of the prodomain and bind in a substrate-like manner along SBT's active site cleft. Residues Y77, A76, H75 and A74 of the prodomain become P1 to P4 substrate amino acids, respectively. These residues conform to subtilisin's natural sequence preferences. The folded prodomain has shape complementary and high affinity to native subtilisin mediated by both the substrate interactions of the C-terminal tail and a hydrophobic interface provided by the β-sheet… The native tertiary structure of the prodomain is required for maximal binding to subtilisin. If mutations are introduced in regions of the prodomain, which do not directly contact subtilisin, their effects on binding to subtilisin are linked to whether or not they stabilize the native conformation. Therefore mutations which stabilize

independent folding of the prodomain increase its binding affinity…

In each claim, the modified prodomain protein also possesses the functional characteristic of increased binding affinity to subtilisin or a variant thereof. As a particular example, the prodomain region of the subtilisin gene from *Bacillus amyloliquefaciens* is utilized. Particular mutations are made to increase the binding affinity to subtilisin ("…binds to subtilisin with ~100 times higher affinity than the wild type prodomain…"; Specification, p. 21). Further, Examples 3 and 5 demonstrate increased affinity of subtilisin prodomain mutants for modified subtilisins.

The examiner alleged that there is no starting point for one of skill in the art to determine a prodomain or variant thereof that has increased binding affinity. Applicant respectfully disagrees. The above-cited portions of the specification provide a structural description of a subtilisin prodomain, an example of isolation of a subtilisin prodomain and examples of mutations demonstrated to increase binding affinity to subtilisin and to subtilisin variants. One of skill is therefore provided not only with a starting point of an isolated subtilisin prodomain, but a description of the structure of such prodomain and guidance regarding amendment of such prodomain.

Claims 3 7, and 13 therefore meet the definiteness requirements of 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

C. Rejection Under 35 U.S.C. §112 – Enablement

In the Office Action mailed August 17, 2009 the examiner has rejected claim 62 under 35 U.S.C. §112, first paragraph as lacking enablement. Specifically, the examiner alleges that "[t]he specification fails to exemplify, describe, or even suggest how to modify any prodomain but a subtilisin prodomain, or how to modify any protease catalytic domain, to achieve the degree of affinity, as measured by dissociation constant, recited in claim 62." (Emphasis added).

In response the examiner's attention is respectfully drawn to section I above. By the present Response, claim 62 has been cancelled. The rejection is therefore moot.

D. Rejection Under 35 U.S.C. §102

In the Office Action mailed August 17, 2009 the examiner has maintained the rejection of claims 1, 46 and 47 under 35 U.S.C. §102 as anticipated by U.S. Patent No. 7,531,325 (previously cited

as U.S. Patent Application No. 2003/0166162), hereinafter "Van Rooijen et al." Applicant respectfully traverses the rejection.

In the Response submitted February 27, 2009 Applicant provided a detailed discussion of the characteristic of "affinity," as used in the claims. Specifically, "affinity" as used in claims 1 and 46 (and claim 47, dependent upon claim 46) describes a subtilisin prodomain protein that can bind tightly and remain bound to a subtilisin. The examiner's attention is respectfully drawn to Section I above, where claims 1 and 46 have been amended to recite a Kd of 10 nM or less. Support for such amendment is found in now-cancelled claim 62 and in the specification at page 18, lines 22-24.

Van Rooijen et al. do not provide a showing of a construct encoding a fusion protein comprising a prodomain protein with a high affinity for a protease. Indeed, Van Rooijen et al. do not describe affinity at all, in particular, not a Kd of 10 nM or less.

The examiner's attention is again respectfully drawn to Example 4 of Van Rooijen et al., describing generation of a GST-cystatin fusion protein, with chymosin propeptides inserted in various locations to form different fusion proteins (shown in Figs. 13-17: GST-KLIP4-Cystatin, GST-KLIP11-Cystatin, GST-KLIP12-Cystatin, GST-KLIP14-Cystatin, GST-KLIP15-Cystatin, and GST-KLIP16-Cystatin). "Table 2 summarizes the cleavage results obtained for each of the corresponding KLIP peptides summarized in FIGS. 13 through 18 expressed in *E. coli*. as GST-KLIP-Cystatin fusion proteins…[p]olypeptide bands corresponding to the molecular mass of free cystatin were subjected to N-terminal sequencing to determine the precise location of the scissile bond…all KLIP-Cystatin fusions were accurately cleaved…" (para. [0133]-[0134]; emphasis added).

As previously detailed in the Response mailed February 27, 2009, chymosin is added to each of the fusion proteins and cleaves the fusion protein at the propeptide sites. The chymosin does not bind to the KLIP propeptide with high affinity, but associates only transiently, which allows release of chymosin, so that it is free to cleave additional fusion proteins. The results of this action are what are shown in Table 2. It is clear from the results (with the exception of KLIP12) that the longer the reaction is allowed to proceed, the more chymosin continues to react with additional fusion peptides in the solution and more free cystatin is generated.

By contrast, if elements with high binding affinity were utilized in the described method, substrate inhibition and poor turnover would occur, and free cystatin would not accumulate beyond the level generated by the first association.

It is well established that high binding affinity is evidenced by a low disassociation constant, as the elements remain bound to one another and do not disassociate. Inversely, low binding affinity is evidenced by a high disassociation constant, as the elements do disassociate. As the chymosin to KLIP disassociation is shown to be high by the accumulation of free cystatin, the binding affinity is shown to be low. It is therefore unclear how the examiner reaches the conclusion that "…the binding and cleavage by a cognate protease...thus is considered to inherently be within the broad range of nM to pM."

Furthermore the fusion proteins produced in Van Rooijen et al. cannot be used in purification methods as described in the present application. The examiner alleged that "Van Rooijen et al. also disclose the use of the prodomain as a separable component for purification of desired fusion partners by affinity chromatography…" Applicant respectfully disagrees with this conclusion.

The examiner cites col. 1-2, 6-9, and 11-17, as well as Figures 2, 3, and 6-12 as demonstrating that the prodomain can be used for purification. No detailed citations are provided to support such conclusion. Applicant notes the discussion of affinity purification in Van Rooijen et al. at col. 6, l. 66 to col. 7, l. 3, where it is stated that "…genes are expressed as fusion products of glutathione-S-transferase (GST), allowing easy purification of the expressed gene from a GST affinity column…" (emphasis added.) This is an affinity purification using standard affinity tag GST. Example 2 provides another example of purification, employing a histidine tag. These examples do not involve proprotein affinity, but affinity to the tag.

At col. 12, ll. 46-49 additional purification methods are described as: "[s]elective binding of the fusion protein to antibodies raised against the pro-peptide sequence and immobilized onto the column, results in selective retention of the fused protein." However, no purification involving the affinity of a prodomain and its associated protease is described in Van Rooijen et al. Due to the transient nature of the binding between KLIP proproteins and chymosin, such affinity purification is not in any way suggested by Van Rooijen et al.

Van Rooijen et al. thus do not provide any showing of a construct encoding a fusion protein comprising a subtilisin prodomain protein with a high affinity for a subtilisin or variant thereof, where the subtilisin prodomain protein binds to a subtilisin or a variant thereof with a Kd of 10 nM or less.

Since Van Rooijen et al. do not describe a construct as set forth in claim 1, 46 or 47, Van Rooijen et al. do not anticipate the claimed invention. Accordingly, withdrawal of the rejection of claims 1, 46 and 47 under 35 U.S.C. § 102(e) as being anticipated by Van Rooijen et al. is respectfully requested.

Additionally, in the Office Action mailed August 17, 2009 the examiner has raised a new ground of rejection, by the rejection of claims 1-3, 5, 12-14, 17 and 46 under 35 U.S.C. §102(b) as anticipated by Ruan et al., *Pro. Sci.*, (1998) 7:2345-2353, hereinafter "Ruan et al." Applicant respectfully traverses the rejection.

Ruan et al. is cited as "meeting the limitations of claims 1-3, 5, 12-14, 17, 46 and 47." Specifically Ruan et al. is alleged to provide a fusion protein comprising a subtilisin prodomain protein operatively linked to a protein of interest in the demonstration of a fusion protein where "the prodomain was synthesized as a fusion protein with the gene III coat protein of the coli phage fd…" The examiner alleged that "the claims require no particular basis for 'interest'…" and therefore the phage protein meets the claim limitation of "a protein of interest." Applicant respectfully disagrees.

It is well established that the scope of the claims should be given "their broadest reasonable construction 'in light of the specification as it would be interpreted by one of ordinary skill in the art.' *In re Am. Acad. of Sci. Tech. Ctr.*, 367 F.3d 1359, 1364 (Fed. Cir. 2004)." (emphasis added; MPEP §2111).

The protein of interest recited in the claims is exemplified in the specification as "a second protein." It is a stated objective of the invention to provide a fusion protein useful in purification, where "[t]he presence of a protease prodomain protein in a fusion protein allows for easy and selective purification of the second protein by incubation with the corresponding protease…" (Specification, p. 14.) By contrast, the phage protein of Ruan et al. was selected

"…so it is displayed on the surface of phagemid particles…" (Ruan et al., p. 2347.) Accordingly, the phage protein of Ruan et al. is not a "protein of interest," as claimed.

Additionally, each of claims 1, 46 and 47 has been amended to recite a functional characteristic of the subtilisin variant, that the variant "retains the activity of subtilisin." Specifically the subtilisin variant must retain the ability to cleave the protein of interest from the complex. Such activity is described in the specification at page 7, lines 30-34 and the Examples.

By contrast, the subtilisin mutant used in Ruan et al. is rendered catalytically inactive ("…an inactive mutant of subtilisin, denoted Sbt15, for use in vitro folding studies…" Ruan et al., p. 2349.) Such mutation was necessary in order to prevent cleavage of the prodomain-subtilisin complex from the phage, which would prevent detection of the binding.

Therefore, Ruan et al. do not describe a fusion protein as set forth in claims 1-3, 5, 12-14, 17 and 46, Ruan et al. do not anticipate the claimed invention. Accordingly, withdrawal of the rejection of claims 1-3, 5, 12-14, 17 and 46 under 35 U.S.C. § 102(b) as being anticipated by Ruan et al. is respectfully requested.

E. Rejection Under 35 U.S.C. §103

In the Office Action mailed August 17, 2009 the examiner has raised a new ground of rejection, and has rejected claims 4, 6-8, 11, 15, 16, and 47-49 under 35 U.S.C. §103(a) as obvious in view of Ruan et al. and further in view of references previously of record: Grøn 1998, Van Rooijen et al. and Grøn 1996. Claim 8 has been cancelled by the present Response. Accordingly, the rejection is addressed below as applicable to claims 4, 6, 7, 11, 15, 16, and 47-49. Applicant respectfully traverses the rejection.

Ruan et al. is cited as in the rejection under 35 U.S.C. §102(b) above and as further "…teaching that the prodomain amino acid substitutions, 'mutations'… 'that stabilize the folding of the prodomain will increase binding to subtilisin…" Grøn 1996 is cited as "teach[ing] the use of modified peptide substrates representing the P4-P3-P2-P1 peptide of a generic, unmodified, prodomain to guide the preparation of amino acid substitutions…" Grøn 1992 is cited as teaching additional subtilisin binding preferences. Further, Van Rooijen et al. is provided as teaching a modified chymosin prodomain adjacent to a desired fusion partner.

In the "Response to Amendment" section of the Office Action mailed August 17, 2009 the examiner stated that "Applicant's arguments are persuasive with respect to the rejection of record of claims herein under 35 U.S.C. §103(a) combining the teachings of Van Rooijen and Grøn et al., of record, and this rejection is RESTATED in further combination in view of the teachings of other prior art of record." Accordingly, it is because of the teachings of Ruan that the prior arguments are deemed to no longer be persuasive.

In sum, the examiner alleges that where the combination of Van Rooijen et al. and Grøn et al. fails to provide a showing of a fusion protein comprising a prodomain protein with a high affinity for protease, citation of Ruan et al. as a primary reference remedies those deficiencies. Applicant respectfully disagrees.

As set forth above, Ruan et al. fail to describe a fusion protein as recited in claims 1-3, 5, 12-14, 17 and 46. Specifically, Ruan et al. fail to describe a fusion protein including "a protein of interest" or a fusion protein that binds to a subtilisin or subtilisin variant that retains the activity of subtilisin with high binding affinity, as is recited in applicant's claims.

Therefore, even if Grøn et al. suggest modification of subtilisin, such modifications were evaluated for the change in k_{cat}/K_M values, and not evaluated for binding affinity, as described in detail in the Response mailed February 27, 2009. Any change in k_{cat}/K_M values is not necessarily predictive of or correlative to a change in affinity.

As detailed above and in the Response mailed February 27, 2009, Van Rooijen et al. provide a complex with a low binding affinity, as demonstrated by the transient association of chymosin and KLIP.

It is understood that "[o]ne cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)." (MPEP §2145) The foregoing discussions of each reference are not meant to attack each reference individually, but are intended to clearly show the deficiencies of the disclosures in the combination of references, since each reference in the combination is required to be considered as a whole.

Based on the foregoing, Ruan et al. in view of Grøn 1998, Van Rooijen et al. and Grøn 1996 fail to provide any logical basis for the construct, fusion protein or method recited in claims 4, 6, 7, 11, 15, 16, and 47-49 and Ruan et al. in view of Grøn 1998, Van Rooijen et al. and Grøn 1996 do not render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 4, 6, 7, 11, 15, 16, and 47-49 under 35 U.S.C. § 103 (a) as being obvious over Ruan et al. in view of Grøn 1998, Van Rooijen et al. and Grøn 1996 is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicant's pending claims 1, 3, 4, 6, 7, 9-13, 15-17, and 46-49 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the August 17, 2009 Office Action without extension was set at three months, or November 17, 2009. This Response is therefore timely and no fees are believed to be due for the filing of this paper. However, should any fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

Date: November 17, 2009

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